AMENDMENT TO THE DRAWING

Please replace Figure 1 with the attached Replacement Sheet for Figure 1.

REMARKS

Claims 1-32 are pending in the present application. Claims 17-32 have been withdrawn pursuant to a restriction requirement. Upon entry of the present amendment, claims 1-16 and claims 33-38 will be pending.

Subsequent to the filing of the previous response on October 19, 2006, the undersigned discovered that claims 1-16 were not, in fact, identical to claims 1-16 of Application Serial No. 10/692,918 ("the '918 application"), as even the Office alleged. Specifically, claims 2 and 4 of the present application included a recitation not included in claims 2 and 4 of the '918 application. The undersigned apologies for this oversight. This supplemental amendment is being filed to replace the prior amendment to effect the correct amendments needed, as well as correct the identifier for claim 11, and indicate amendments to claim 7 that were not indicated previously. Applicants request that the response filed on October 19, 2006 be replaced with the present response, but that all the papers submitted with the prior response be utilized as if filed with the present response.

Claims 1 and 3 have been amended herein to recite that the single heavy chain antibodies are produced in response to antigen challenge. Support for this amendment can be found, *inter alia*, on page 16, lines 16-19, page 31, line 31, through page 32, line 3 and claim 31 of the application as filed. Applicants respectfully submit that, contrary to the arguments made by the Office regarding claims 17-32, i.e., that the products could be made by another process, such is not the case for the antibodies produced according to the methods of claims 1 and 3 as amended. Riechmann et al, 1999, cited by the Office, does not disclose or suggest that antibodies may be derived from transgenic animals in response to antigen challenge. The work of Riechmann is focused on the derivation of engineered VH binding domains based

on a combination of phage display of human or camelid VHH domains and site directed mutagenesis to improve the stability and solubility of VH domains in the absence of a variable light chain domain (see Davies and Riechmann(1995) *Biotechnology* 13, 475-479). This approach lacks the advantages of natural processes which provide for the derivation of high affinity antibodies as a result of "class switching and somatic hypermutation (affinity maturation) which is necessary for the production of specific antibodies which bind to their antigen with selectivity and high affinity." (*See* page 5, lines 16-24, of the application as filed.) Thus, Applicants request that the restriction requirement be withdrawn in view of the amended claims.

Claims 1 and 3 have also been amended to specify that the mammal is non-human. Support for this recitation can be found, *inter alia*, on page 15, lines 27-31, of the application as filed.

Claims 1 and 3 have also been amended to recite that the locus is expressed specifically in B cells. Support for this amendment can be found, *inter alia*, on page 5, lines 24-28; page 6 lines 11-17; page 23 lines 8-14, and page 24, lines 6-9, of the application as filed.

Claims 1 and 3 have also been amended to remove the recitation regarding a recombination sequence "rss" as this recitation was for a preferred embodiment regarding claims comprising Cγ constant heavy chain genes only.

Claims 2 and 4 have been amended to remove the recitation that the heavy chain genes do not express a functional CH4 domain. Support for this amendment can be found, *inter alia*, on page 24, lines 17-21, of the application as filed, wherein it is disclosed that the CH1 **and/or** CH4 domains are rendered non-functional. Further, Applicants direct the Office

to page 8, lines 9-16, of the GB priority application filed April 24, 2001, incorporated by reference upon the filing of the present application. Rendering only the CH1 domain nonfunctional is discussed. It is clearly not necessary to render the CH4 domain non-functional. Moreover, some classes of antibody, such as IgG, do not naturally comprise a CH4 domain. Concomitantly, claims 1 and 3 have been amended so as not to limit the claims to the VHH and camelised VH antibodies as defined in the specification, to the extent the same are interpreted to be required not to have a functional CH4 domain.

Claims 2 and 4 have been further amended to clarify that the expression of the locus leads to the formation of antibody. Support for this amendment is implicit from an understanding of basic molecular biology.

Claims 2 and 4 have also been amended to remove the recitation of "complete" before single chain antibody. Support for this amendment can be found, *inter alia*, on page 20, lines 14-20, of the application as filed.

Claim 4 has amended to recite that the VH region is mutated such that the single heavy chain antibody is stabilised. Support for this amendment can be found, *inter alia*, on page 3, lines 10-17, of the application as filed.

Support for new claims 33-36 can be found, *inter alia*, on page 10, line 19, through page 11, line 11, page 12, line 21 through page 13, line 2, and page 13, line 27, through page 14, line 19, of the application as filed.

Support for new claim 37 can be found, *inter alia*, on page 16, lines 7 through 9, and page 30, line 5 through page 32, line 13, of the application as filed. Support for a new claim 38 can be found, *inter alia*, on page 16, lines 7-12 and page 29, line 6 through page 30, line 2, of the application as filed.

Other amendments have been made to change dependencies or include recitations from other claims.

Preliminarily, Applicants note that a copy of the Great Britain priority application filed on April 24, 2001 was forwarded under separate cover on September 26, 2006. Applicants request confirmation of its receipt.

Further, the Office requested a corrected Figure 1. A Replacement Sheet for Figure 1 is attached to this response.

Finally, the Office objected to the disclosure regarding the reference to a Figure 3 in the specification, and for allegedly containing an embedded hyperlink and/or other form of browser-executable code. The specification has been amended to delete the reference to Figure 3 and the reference to the hyperlink.

Applicants feel a brief summary of the invention, and the background leading to it, may be instructive. As set forth in the application as filed, camelids contain, in addition to normal antibodies containing heavy and light chains, single chain antibodies containing only heavy chains that bind antigen with high affinity. The single heavy chain antibodies are encoded by a distinct set of VH (variable heavy chain) segments referred to as VHH genes. Both VH and VHH are interspersed in the genome, and use a common D segment. The camelid single heavy chain antibodies are also missing the entire CH1 domain in the constant region of the heavy chain. In the camelid, the exon coding for CH1 is present in the genome, but is spliced out due to the loss of a functional splice acceptor sequence. (See page 2, line 21 through page 3, line 9, of the application as filed.)

As was known in the art at the time of the priority date of the present application, only IgG_2 and IgG_3 single heavy chain antibodies are produced in camelids. (*See* Hamers-

Casterman *et al.*, Nature, 363:446-448, 1993.) As of the priority date of the present application, development of B-cells expressing VHH camelid antibodies was not understood. (*See* PCT/GB2003/005274 (copy enclosed) and the De Genst et al reference cited by the Office.) It was not known whether the IgM pathway was circumvented, or whether there was a specialist camelid lymphocyte sub-population. It was also not known whether specialist camelid specific factors were necessary to process, assemble and express VHH single chain antibodies.

Published work was very limited. It was known that mammalian cells in culture can express and secrete a rearranged camelid VHH heavy chain only antibody derived from mRNA, and that VHH domains and fragments thereof can be expressed in bacteria. This information supported earlier published work which demonstrated that functional human VH domains can be selected from randomized phage display libraries (*see* Ward et al. (1989) *Nature*, 341, 544-546, copy enclosed) and that rearranged heavy chain genes engineered to be devoid of CH1 (and mutated to prevent membrane capture) can be expressed and secreted by mammalian cells in culture (*see* Sitia et al (1990) Cell, 60, 781-790, copy enclosed). PCT/GB2002/02867 (copy enclosed) describes the attempted expression of heavy chain only antibody in which the light chain locus was deleted; the productive expression of heavy chain only antibody was not achieved, however.

In contrast to the uncertainty of the prior art, the present invention discloses a method for the expression of single VHH or camelised VH heavy chain antigen specific antibodies comprising heavy chain regions devoid of CH1 in transgenic animals in response to antigen. VDJ rearrangement and somatic hypermutation is disclosed in response to an antigen challenge in the absence of light chain gene expression. Moreover, monoclonal antibodies

can be derived using well established technology and antigen specific heavy chain only antibodies can be generated.

Rejection for Statutory Double Patenting

Claims 1-16 were provisionally rejected under 35 U.S.C. 101 as claiming the same invention as that of claims 1-16 of copending Application No. 10/692,918. This rejection has been overcome by the amendments to claims 1 and 3. Applicants request that this rejection be withdrawn.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1-16 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement. Applicants traverse this rejection.

The Office initially asserts that the aspects considered broad are 1) breadth of subject population (alleged to be any mammal) for expressing VHH heavy chain locus, 2) any vector for cloning megabase transgene, and 3) using ES cells of any species and expressing VHH locus. These assertions are either not correct, or irrelevant.

First, as clarified in the present claims, the breadth of the subject population is not any mammal, as the Office contends, but non-human mammals. Second, the Office's allegations that the transgenes are megabase transgenes is incorrect. As noted on page 24, lines 25-27, the loci of the invention are generally 200-250 kB in size. Contrastingly, megabase is defined in the art as 1 million nucleotides. See, for example, http://en.wikipedia.org/wiki/Megabase. Finally, ES cells are not required to practice the invention. As set forth on page 27, line 24, through page 28, line 6, in addition to using ES cells, the loci can be integrated into a recipient mammal using microinjection into a fertilised egg, or nuclear transfer using any cell initially, and transferring the nucleus of the

transformed cell to a fertilized egg. Indeed, as will be discussed in more detail below, Applicants have prepared transgenic mice using microinjection.

More specifically, the Office contends that the specification provides only prophetic and general methodology without disclosing specifics. The Office is reminded that working examples are not required. The Office further contends that sequence information is not provided for the YAC or BACs, and that the specification fails to provide any sequence information for the vectors that would be required for the multi step cloning process. But, sequence information is not necessary for the YAC or BACs; the invention is not limited to a particular vector, nor has the Office provided evidence that it should be. Nor is the sequence for a particular vector necessary. All that is necessary to be shown are the components; Figure 1 and the specification amply disclose this, including additional sites/regions that are contemplated to be beneficial, such as FRT (see page 21, lines 16-19), LoxP (see page 21, lines 20-23), and immunoglobulin LCRs (see page 23, lines 12-14). It is noted that the claims, as amended, recite that the expression is B-cell specific. Cell specific transgene expression in transgenic mammals was an established technology as of the priority date of the present invention -- LCR elements were known to provide integration site independent, tissue specific expression of the incorporated transgene in mammalian genomes (*see* Grosveld et al, (1987) *Cell*, 51, 975-985, copy enclosed). An immunoglobulin LCR was available as of the priority date, and is referenced in the application as filed (*see* Li et al, *Trends in Genetics* (1999) 15(10), 403-408, Table 1, copy enclosed) . This enables expression of the VHH or camelised VH locus in B-cells. Indeed, as is clear from the enclosed recent internet-published paper, Applicants have generated both IgG and IgM heavy chain only antibodies in mice following the disclosure of the specification as filed. See Janssens et al, *PNAS*,

103:15130-15135, October 10, 2006, copy, with supplementary materials, enclosed.

Specifically, the cited paper discloses the expression of loci containing IgM and IgG, and IgG only, human constant regions, lacking CH1, with two camelid VHH regions, and human D and J regions in mice. Bac clone 11771 and pFastBac were both used successfully. The loci further contained FRT and LoxP sites, and immunoglobulin LCR. The vectors were injected into fertilized mouse eggs of animals that do not produce surface IgM and have a block in B cell development at the pre-B cell stage. The mice containing IgM and IgG constant regions produced IgM; the mice containing IgG constant regions produced IgG. The mice were then injected with antigen – *E. coli* heat shock protein 70; a combination of *Diphtheria* toxoid, whole cell lysates of *Bordetella pertussis*, *Tetanus* toxoid, and inactivated poliovirus; rtTA (the IgG only mice) and TNFα (IgM and IgG containing mice). Antibodies of the desired specificity were obtained. The preparation of hybridomas therefrom, i.e., by isolating the spleen of the mice, in addition to polyclonal antibodies, is also reported. Although Applicants contend that the submission of this peer-reviewed publication is sufficient to evidence enablement, Applicants reserve the right to submit a declaration providing the same information if necessary.

The Office argues that prior art suggest that the repertoires of camelised VH domains were initially created by randomization of residues within the third hypervariable loop H3. As discussed above, the prior art is limited to the derivation of camelised VH domains or camelid VHH domains using a combination of phage display of randomized camelid VHH or human VH domains. These were engineered to incorporate three camelid specific mutations to improve the stability of the resulting VH domains in the absence of a stabilizing variable region supplied by the immunoglobulin light chain. (*See* Davies and Riechmann (1995)

Biotechnology, 13, 475-479, and Davies and Riechmann (1996) *Immunotechnology* 2, 169-179, copies enclosed). Spinelli et al (1996) *Nat. Structural Biol.*, 3(9), 752-757 (copy enclosed) describes structural changes which stabilise the camelid VHH in the absence of light chain and compare this with the human VH, which differs by four amino acids (see page 3, lines 10-17). The prior art, thus, describes how best to stabilize (camelise) a V gene such that when expressed as a VH domain, in the absence of light chain, stability and solubility are enhanced. It does not, however, teach the derivation of antigen specific high affinity antibodies in transgenic animals in response to antigen challenge.

Applicants respectfully request that this rejection be withdrawn.

Rejection Under 35 U.S.C. § 102

Claim 1 was rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Ledbetter et al (WO 99/42077). Applicants traverse this rejection.

Claim 1 has been amended herein to recite that the antibody is expressed specifically in B cells. Ledbetter et al does not disclose or suggest this element of the claims. Neither is Ledbetter et al enabling for what it purports to disclose.

Applicants respectfully request that this rejection be withdrawn.

Rejections Under 35 U.S.C. § 103

Claims1-4 and 7-16 were rejected under 35 U.S.C. § 103(a) as allegedly obvious over Lonberg et al (U.S. Patent No. 5,625,126) and Riechmann et al, 1999, *Immunological Methods*, 231(1-2):25-38). Applicants traverse this rejection.

Applicants observe that, although the Office alleges that the level of the skill in the art renders the invention unpredictable, it argues the contrary in support of this rejection. Specifically, on page 14 of the Office Action, the Office argues that

> in view of the high level of skill in molecular biology techniques at the time of filing, one of the [sic] ordinary skills [sic] in the art would expect a reasonable expectation of success in modifying the transgene to include VHH/camelised VH region in the method disclosed in Lonberg ('126) for producing heterologous antibody in mouse.

The Office further stated it would only require routine experimentation to replace human VH to VHH disclosed by Riechmann. Regardless, as discussed in the summary above, there was no reasonable expectation of success of producing single chain antibodies having a camelid VHH or camelised VH in a transgenic animal for reasons set out in PCT/GB2003/005274, which was **after the filing of the present application disclosing how to do so.**

Further, the claims as amended recite that the antibody is produced in response to antigen challenge. Neither reference relied upon by the Office discloses or suggests this limitation.

Applicants respectfully request that this rejection be withdrawn.

Claims1-16 were rejected under 35 U.S.C. § 103(a) as allegedly obvious over Lonberg et al and Riechmann et al, further in view of Green et al. (U.S. Patent Application No. 20030093820). Applicants traverse this rejection.

Again, the high level of skill in molecular biology is cited. The deficiencies of Lonberg et al and Riechmann et al, however, are discussed above, as was the view of those of skill in the art. Green et al, is not an appropriate reference. Green et al claims priority to a PCT application filed June 8, 2000.

> For U.S. application publications of applications that claim the benefit under 35 U.S.C. 120 or 365(c) of an international

> application filed prior to November 29, 2000, apply the reference under 35 U.S.C. 102(e) as of the actual filing date of the **later-filed** U.S. application that claimed the benefit of the international application.

MPEP 2163.03, emphasis added. The effective filing date of Green et al, thus, is November 30, 2001, after the priority date of April 24, 2001 of the present application.

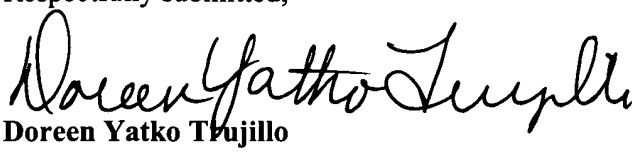
Regardless, Green et al does not provide any motivation to combine with Lonberg et al or Riechmann et al, or *vice versa,* nor does it overcome the failure of these two references to establish a reasonable expectation of success, particularly considering the more relevant camelid art, discussed above.

Applicants respectfully request that this rejection be withdrawn.

CONCLUSION

Applicants respectfully submit that claims 1-16 and 33-38 are in condition for allowance. An early notice of the same is earnestly solicited. The Examiner is invited to contact Applicants' undersigned representative at (215) 665-5593, if there are any questions regarding Applicants' claimed invention.

Respectfully submitted,



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